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MESENTERIC TUMOR ASSOCIATED WITH PREGNANCY

With Report of a Case

ERLE HENRIKSEN, M.D.

From the Gynecological Department of the Johns Hopkins Hospital and the Johns Hopkins University

Submitted for publication June 8, 1936

Tumors of the mesentery, either cystic or solid, are rare enough to warrant a brief report, especially when such a tumor is associated with a normal pregnancy. These neoplasms are so infrequently encountered that many textbooks and systems of surgery make only brief reference to them or omit mention of them altogether. Rankin and Majors[1] (1932) reported 22 tumors of the mesentery from the Mayo Clinic and, of these, 7 were benign cysts. However, the literature concurs with their opinion that the cystic type is more common than the solid, and that the incidence is equally divided between the sexes. The variations in size, the varied distribution and the confusion of pathological interpretation all tend to complicate the controversy pertaining to their origin, which, as with many rare conditions, is a matter of conjecture. The reported series are, as a rule, too small to permit any satisfactory conclusions on this subject.

CASE REPORT

E. E., white woman, aged 32, was admitted to the Gynecological Service because of a palpable mass in the left upper quadrant. The history revealed normal pregnancies in 1933 and 1934 with a spontaneous abortion in the second or third month of pregnancy in October, 1935. The patient became pregnant for the fourth time two months after this abortion. There were no gastro-enteric or genito-urinary symptoms. For a few months, however, the patient had complained of tiring easily on moderate exertion. No loss of weight had been noted.

Present illness. Two days following the last normal period and 13 weeks prior to admission, the patient noted a freely movable, firm, non-tender mass

[1] Rankin, F. W., and Majors, S. G., Tumors of the Mesentery. *Surg., Gynec., & Obst.*, 1932, **54**: 809.

"about the size of a small orange" in the left upper quadrant. There had been a slow but gradual increase in the size of the mass. There were no associated gastro-enteric symptoms.

Examination. This was essentially negative except for the presence of a firm, rounded smooth mass, about 12 centimeters in diameter, which was movable and could be readily and painlessly pushed downwards and in an arc to the right lower quadrant. On release of pressure, the tumor mass immediately returned to the left upper quadrant. Pelvic examination revealed a boggy uterus enlarged to the size of a two or three months' pregnancy; several small myomatous nodules were outlined on the posterior surface. The laboratory findings were essentially normal. Roentgenograms revealed a shadow in the left upper abdomen which was interpreted as a possible tumor mass. Various pre-operative diagnoses were made and among these the possibility of mesenteric cyst was included.

Operation. In view of the gradual increase in size of the abdominal mass of uncertain origin an operation was thought advisable in spite of the presence of the uterine pregnancy. Under avertin-ether anesthesia the abdomen was entered through a midline incision. The uterus presented a typical appearance of a 2½ months' pregnancy, with several small myomatous nodules present on the posterior surface. The adnexa were normal. Lying between the layers of the jejunal portion of the mesentery was a firm rounded mass about 12 centimeters in diameter. The tumor was easily moved about the abdomen and could be manipulated within the limitations of the mesentery. The mass approached to within 3 centimeters of the mesenteric attachment. In the latter region were several enlarged lymph nodes; frozen sections of these revealed normal lymphoid tissue. An attempt was made to decapsulate the tumor, but this was unsuccessful because of the distribution of the large blood vessels over the surface. The tumor was then removed *in toto*, together with 50 centimeters of intestine, care being taken to conserve the circulation of the remaining intestine; a lateral anastomosis was then made.

The patient was given nothing by mouth for 5 days, fluids being supplied intravenously in the form of glucose and hypertonic saline. The postoperative course was smooth and the patient was discharged without complaints on the 16th day after the operation.

Subsequent roentgenographic studies showed an excellent anastomosis. The pregnancy was continuing its normal course at the end of the 6th month.

Pathological note. The specimen consists of 50 centimeters of normal-appearing small intestine. Lying between the sheaths of the mesentery is a firm, rounded tumor mass measuring 8.5 x 8.5 x 8 centimeters in diameter. There are several small lymph nodes present near the site of attachment. On section, the gross picture is that of a benign uterine myoma. Microscopically, the tumor is a typical benign myoma. However, the fibers and stroma are swollen and the nuclei are very vesicular and stain irregularly—the histological picture seen in uterine myoma with associated pregnancy.

COMMENT

A review of the literature reveals no constant symptom or symptom complex which would be of value for the diagnosis of the majority of these tumors. Movability, usually in an arc corresponding to the

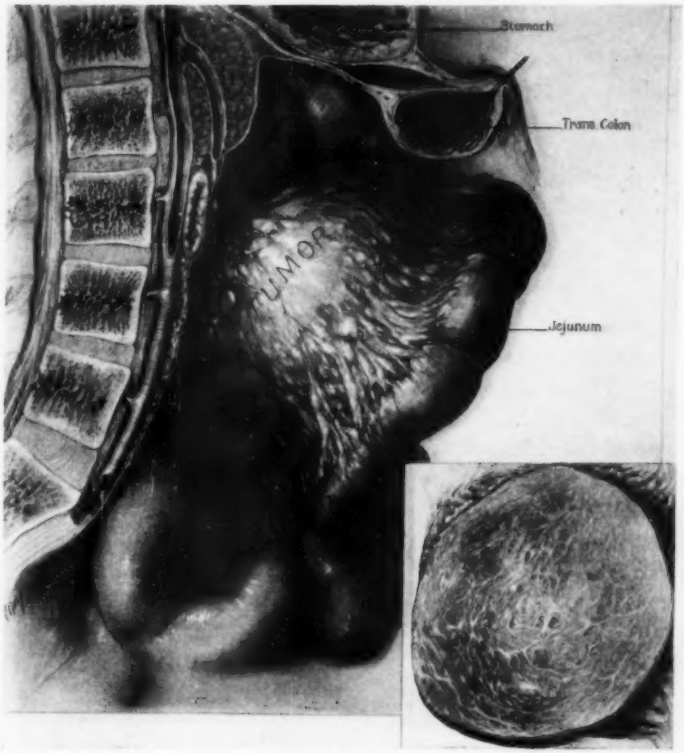

FIG. 1. TUMOR GROWING BETWEEN THE LAYERS OF THE JEJUNAL PORTION OF THE MESENTERY. INSET: CROSS SECTION SHOWING THE TYPICAL APPEARANCE OF A BENIGN MYOMA

mesenteric axis, is the most dependable diagnostic aid. The ease or difficulty of the diagnosis is naturally dependant upon the size and location of the tumor. The jejunal portion of the mesentery is an exceedingly rare location for these tumors. Tumors of other organs

must be eliminated, either clinically or with the aid of roentgenographic studies. In most instances the development of the tumor is insidious and the clinical symptoms it produces are aggravated as the size of the tumor increases. If it is large, pressure on the adjacent viscera may cause gastro-enteric disturbances. The mobility of the tumor tends to twisting and may produce torsion of the intestines or blood vessels, such occurrences being associated with acute abdominal symptoms. Diagnosis in the acute instances is practically impossible, and even in the uncomplicated cases it is apt to be very difficult.

While in this instance the patient became aware of the tumor following conception and while the microscopical picture shows cellular changes like those encountered in benign uterine myomata associated with pregnancy, we believe that the pregnancy had no part in the origin of the tumor.

TREATMENT

The *treatment* of mesenteric tumors cannot be outlined dogmatically. The size, the location, the suspected histological nature of the tumor and the condition of the patient must all be considered. When the condition of the patient permits and the anatomical hazards are not too great, the tumor should be removed. In most instances of a primary mesenteric tumor, resection of the bowel has been necessary. If the surgical approach is contraindicated, either because of the location or the nature of the tumor, radiation therapy, though of unknown value, may be resorted to. Of paramount importance in every instance, however, is an exploratory operation to establish the diagnosis.

SUMMARY

1. Mesenteric tumors present no characteristic symptom complex and must be diagnosed by elimination of tumors of other organs in the region of the mesentery. Movability in an arc corresponding to the axis of the mesentery is the most common and distinctive finding.

2. Because of the variation in size, the varied distribution and the great confusion in pathological interpretation, the etiology of these tumors is controversial.

3. The normal uterine pregnancy in this case apparently played no part in the origin of the tumor.

4. The treatment of mesenteric tumors is dependent upon the site, the pathological nature of the tumor and the condition of the patient. In every instance an exploratory operation is indicated.

We are indebted to Dr. Thomas S. Cullen and Dr. Fred Geib for their assistance. We also wish to express our gratitude to Miss Marjorie Hoag for the illustration.

JAUNDICE

Methods of Diagnosis and Treatment of Its Causes

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Submitted for publication July 27, 1936

In discussing the subject of jaundice the tendency is to overemphasize the chemical approach, and to fail to develop the clinical side. There is little doubt that a knowledge of biochemistry is becoming more and more essential to an understanding of Modern Medicine, and it would be difficult to exaggerate how much of the elucidation of the pathology of jaundice has come through physiological chemistry. Nevertheless, jaundice is far from being a completely solved subject, and as yet the advances on the biochemical side should be utilized only in accordance with the judgment of clinicians. One may be thoroughly appreciative of the tremendous advances made by the biochemists, and still recognize their limitations and their fields of application. From partially completed or from poorly understood studies ill advised conclusions are liable to be reached by the clinician.

Let us suppose a clinician or a biochemist finds some clinical application for his laboratory studies. It is asking almost too much of human nature for him not to be slightly enthusiastic over the importance of his data. Another and another man make contributions on the same subject, but by slightly different approaches. They in turn publish their data, and their ideas on their clinical use. The subject soon becomes complicated and difficult to handle. In the field of liver and bile-duct pathology we have an excellent example of such a condition. Glucose tolerance tests, galactose tolerance tests, load bilirubin tests, serum retention dye tests, Van den Bergh tests, icterus index estimations, red blood cell fragility, reticulocyte counts, red blood cell diameter measurement, cholesterol-free and ester-form estimations, repeated diagnostic non-surgical biliary drainages, gastric

analyses, urobilinogenuria, stool examinations for urobilin, gastrointestinal and gallbladder x-ray studies, estimation of urinary tyrosine, glycine fixation of benzoic acid with hippuric-acid formation, and its estimation in the urine, urinary protease studies, and the estimation of bile acids in the urine, have all been suggested as helpful or essential procedures in the establishment of the etiology of jaundice. Truly an imposing array!

CLASSIFICATION

In recent years it has become the custom of several writers to make a clinical division of jaundice into two classes, painful and non-painful. Behind this conception is apparently the thought that the colic is generally due to calculus formation, and that patients with colic are usually subjects for operation, whether they have stones or not. In general, this is a practical working rule.

In the group of "painless jaundice" are included the cases of hepatitis, silent common-duct stone, and carcinoma of the head of the pancreas. A few other rarer conditions could also be included. It is felt that for this group a correct diagnosis is difficult, but all the more important, since the treatment of the first condition is medical, while that of the second and third is surgical. It is apparently tacitly agreed that the cases of hepatitis and of carcinoma of the head of the pancreas are without pain. This is true in a number of instances, but the fallacy of making such a classification lies in the fact that a significant proportion of patients in these two groups do have pain (1) (2). In cases of carcinoma pain is particularly important, for it is deep-seated and boring, simulating that of common-duct stone when it is below the level of colic. In hepatitis pain, although of a lesser degree, is often present.

There is another group of cases which could be called painless, in which the depth of the icteric tinge is less than in those listed above, and in which the jaundice is caused by an entirely different mechanism. This group will be described below as representing cases of retention jaundice.

There is one classification, an anatomical one proposed by Rich (3), which is simple and would seem to be accurate. It has the further advantage of utilizing a simple stable basis from which one may

feel one's way to possible resulting clinicopathological states in an orderly manner, instead of trying to pin down a diagnosis on such an unstable symptom as pain, which may be an expression belonging to any number of basic lesions.

The basis of this classification is as follows:

I. Jaundice in association with intact bile canaliculi: Retention.

II. Jaundice in association with ruptured bile canaliculi: Regurgitant.

I. In the first group the jaundice is due to the retention of bilirubin within the blood vessels. In theory this may be due to greatly increased bilirubin production, for instance, from increased red blood cell destruction; from a postulated, but unproved, elevated hepatic excretory threshold for bilirubin; from a decreased functional ability of the liver parenchymal cells to excrete bilirubin, thereby simulating the condition of the kidney during anuria—also unproved. However, it seems most likely, as has been indicated and demonstrated by Rich, to be due to a combination of increased bilirubin production together with liver-cell damage without rupture of the bile canaliculi. So great is the reserve belonging to the liver that increased destruction of red blood cells would have to be excessive to be the sole cause for the jaundice. The conditions commonly seen in man which may be productive of the retention type of jaundice are: hemolytic jaundice, sickle-cell anemia, malaria, paroxysmal hemoglobinuria, lobar pneumonia, myocardial insufficiency, pulmonary infarcts, hemolytic septicemias, icterus neonatorum and pernicious anemia.

This first group is fundamentally different from the second, in that the cells lining the bile canaliculi are intact and the bilirubinemia differs only in degree from that noted in normal individuals. It is demonstrated as an indirect Van den Bergh reaction. Bilirubin in these cases is not demonstrable in the urine. In certain stages of these diseases the Van den Bergh reaction may become direct. This would indicate a necrosis of the parenchymal cells of the liver and a passage of bile back into the blood or lymph.

II. To the second group in the classification of jaundice on an anatomical basis belong cases produced by conditions that cause discontinuity of cells lining the bile canaliculi. It may be produced by various factors:

1. Obstruction of the common or the hepatic duct.

2. (a) Necrosis of parenchymal cells of the liver by infection.
   (b) Necrosis of parenchymal cells of the liver by toxic agents.

There are various subdivisions of these two headings which may be described as follows:

1. Back pressure from common or hepatic duct obstruction due to:
   Tumor of the head of the pancreas.
   Tumors in the ductus choledochus.
   Extra-biliary tumors in the region of the ductus choledochus; glands, gumma, neoplasm, etc.
   Pressure from small intestinal diverticula acting on the ductus choledochus.
   Choledochus-duct or multiple hepatic-duct stones.
   Pericholedochus-duct adhesions.
   Other rare conditions—parasites, retroperitoneal tumors, aneurysms, etc.

Except in the case of calculi, encroachment of the other conditions on the duct is gradual. The intrahepatic bile channels can slowly accommodate themselves to the increasing back pressure and become dilated. As the obstruction becomes more complete, there is greater and greater pressure on the polygonal liver cells and eventually they separate one from the other and bile is allowed free access to the lymphatic and blood vessels. In time a certain number of polygonal cells are destroyed by pressure and the bile has an even freer access to the general circulation. Consequently, in these later stages, the mechanical distinction between the obstructive and the parenchymal types of jaundice becomes notably less. This should be borne in mind in considering the basis for the use of such tests as galactose tolerance, which will be mentioned later.

2. *Parenchymatous liver changes*—Hepatitis and cholangeitis.
   a. Infectious: Catarrhal jaundice (etiology unproved).
      Weil's disease—Spirochetosis (Leptospira) ictero-haemorrhagica.
      Multiple foci of infection.
      Portal pyelothrombosis.
      Yellow fever.
      Syphilis (congenital).
      Biliary cirrhosis (etiology unproved).
   b. Toxic: Acute yellow atrophy.

Phenylcinchoninic acid derivatives.
Carbon tetrachloride.
Arsphenamine.
Chloroform.
Trinitrotoluene.
Phosphorus.
Portal cirrhosis.
Mushroom poisoning.

In these conditions, which are directly responsible for the destruction of polygonal cells of the liver the production of jaundice is a simple affair. In the early stage, jaundice may be due to hyperbilirubinemia as a result of lowered liver efficiency. As the disease progresses and cells are destroyed, the type of jaundice changes to one produced by the direct flow of bilirubin and other products of bile, such as cholesterol and bile salts, into the blood and lymph circulation.

Jaundice of this character need not be associated with the absence of bile in the stool and in fact in a large percentage of the cases, especially of toxic hepatosis, there is merely a decrease in quantity.

Van den Bergh Test (*4*)*:* At this time it would seem fitting to outline the work which has demonstrated the difference between the direct and indirect Van den Bergh reactions. This distinguished investigator found that when Ehrlich's diazo reagent was added to the serum of individuals with obstructive jaundice a prompt reaction took place, with the formation of a pink to a lavender color (this color range has since been shown to be a factor in the hydrogen-ion concentration). He called this a *direct* reaction. He also found that bilirubin which had not been excreted by the liver gave a reaction only after the serum proteins had been precipitated by alcohol. This he called an *indirect* reaction. Subsequent investigations showed that there were numerous conditions in which this type of reaction was demonstrable.

The work of Barron (5) indicates that the addition of a surface-tension-reducing substance to serum will release bilirubin from the serum proteins and cause it to unite directly with the Ehrlich reagent. Just how bilirubin is freed from its protein combination by the liver cells and excreted into the bile is not known, but when this free bili-

rubin is reabsorbed into the blood stream in cases of regurgitant jaundice, it is accompanied by bile salts and cholesterol and perhaps by other substances which lower the surface tension of the serum sufficiently to permit the bilirubin not only to unite directly with the Van den Bergh reagent but, what is more of a natural phenomenon, to be excreted by the kidney, an organ which does not have the ability to break down the protein-bilirubin complex and excrete bilirubin normally into the urine.

Second only in importance to the bilirubin circulation, is that of urobilinogen (6) (7). When bilirubin reaches the intestine, it is acted upon by bacteria and reduced to urobilinogen. This in turn is partly excreted by the bowel and partly absorbed by the blood. In its course through the portal circulation a part of it is excreted by the liver, and as the remainder of it gains the general circulation, it is passed out through the kidneys.

If there is an increased amount of bilirubin formation from excessive red blood cell destruction, an increased formation of urobilinogen follows. The reabsorbed portion will go through the pathways mentioned above. If, however, the liver is damaged, it is unable to excrete even its normal load of urobilinogen and the retained amount is filtered out through the kidneys.

This knowledge has been put to valuable clinical usage. If there is no urobilinogen or its oxidized product, urobilin, in the urine or stool, one is safe in assuming that no bile is reaching the intestine. This may be due to an obstruction or to the cessation of bile secretion by a badly damaged liver. There are two instances, occurring rarely, in which urobilinogen may be demonstrable in spite of complete biliary obstruction:

1. If the bile above the obstruction becomes infected, the bacteria may reduce bilirubin to form urobilinogen. This in turn may be absorbed and be found in the urine.
2. In cases of long-standing complete biliary obstruction, bilirubin may be secreted through the wall of the gut. A portion of this will naturally be reabsorbed and excreted into the urine. The remainder will be found in the stool.

If there is constant or periodic urobilinuria, bile is reaching the intestine in a steady stream or in spurts, for instance, around a com-

TABLE I

Bilirubin and urobilinogen in various pathological states

DIAGNOSIS	PATHOLOGICAL CLASSIFICATION OF BILE CANALICULI	MECHANISM OF JAUNDICE	BLOOD BILIRUBIN	URINE		STOOL, UROBILINOGEN
				Bilirubin	Urobilinogen	
Normal	Intact		±	0	+	+
Decreased liver efficiency without increased blood destruction						
Anoxemia	Intact	No jaundice	±	0	++	+
Mild hepatitis as in cholecystitis, pregnancy, etc.						
Decreased liver efficiency with increased blood destruction						
Pernicious anemia	Intact	Retention	Indirect ++	0	++	++
Hemolytic jaundice						
Sickle cell anemia						
Myocardial insufficiency						
Pneumonia						
Malaria						
Prolonged febrile states						
Necrosis of liver cells from toxin and infection:						
1. Bile reaching intestine Any type	Ruptured	Leakage of liver bile into blood stream	Direct or biphasic + to ++++	+	+++	+
2. Bile not reaching intestine Any type	Ruptured	Leakage of liver bile into blood stream	Direct or biphasic + to ++++	+	0	0
Obstruction						
1. Partial	Ruptured	Leakage of liver bile into blood stream	Direct or biphasic + to ++++	+	++	+
2. Complete	Ruptured	Leakage of liver bile into blood stream	Direct or biphasic + to ++++	+	0	0

mon-duct stone or during stages in the course of a catarrhal jaundice. Urobilinogenuria can generally be demonstrated during the course of the latter condition, if it is looked for two or three times daily. During the average course of a mild toxic hepatitis, as a rule bile is constantly secreted and reaches the intestine. It is only in the most severe (and usually terminal) stage of the process that urobilinogen cannot be found in the stool and urine in increased amounts. The relationship between bilirubin and urobilinogen is outlined in Table I.

DIAGNOSTIC PROBLEM

It was said above that an excessive number of tests have been advocated for the demonstration and estimation of liver pathology. It is natural that an organ having the protean activities of the liver should lend itself to various types of study, and in the interest of medicine it is proper that these and also future investigations should be made. However, as it is essential, both from the point of view of fellow feeling and medical judgment, that the patient be subjected to the least possible disturbance, it is wise from time to time that an estimation be made of the practical clinical value of the different tests. Only in this way can certain of them be selected and used intelligently.

Jaundiced patients are frequently sick individuals and are fervently hopeful and appreciative of being let alone. Consequently, it is fortunate that in the majority of cases their illness can be diagnosed by such simple means as history, physical examination, and the more common routine laboratory examinations which should include Van den Bergh tests and determinations of urobilogenuria and bilirubinuria.

Retention Jaundice: Several writers have called attention to an elevated bilirubinemia of the indirect type not associated with demonstrable cirrhosis, hemolytic jaundice, pernicious anemia or any recognizable infectious process. The condition has occasionally been noted in individuals who were undergoing a routine check up. Schiff (8) mentions it, Rozendaal and others (9) have reported a number of cases, and the author has found several in his own practice. So far there are no data from which one can establish the etiology. Rozendaal and his co-workers (9) believe it due to a dysfunction in the liver

excretory threshold for bilirubin, and not to damaged polygonal cells. This is a possible explanation, but to date purely theoretical.

Rous and his co-workers (10) and Mann (11) have shown that when two-thirds of the liver is removed, the remainder is apparently sufficient to carry on the metabolic processes normally required of the whole liver. These include the excretion of the normal amount of bilirubin, and if but one-third of the cells are left, this signifies that they are capable of at least tripling their normal duties. In recognition of this extensive reserve, it seems that one would be a little premature in believing that an increased hepatic threshold is the basis for these unexplained cases of hyperbilirubinemia.

These cases are interesting and their pathological changes may be important. It would appear to be the better part of wisdom to consider them as presenting an unsolved problem, rather than labeling them as examples of liver dysfunction and so running the risk of pigeon-holing them.

Among the diseases in which an indirect Van den Bergh reaction is found, *hemolytic jaundice* (familial or acquired) is at times difficult of diagnosis. For its positive identification one must demonstrate an increased fragility of the red blood cell, an increased reticulocyte count, a diminution in the red blood cell diameter and an enlarged spleen. There are cases in which any of these findings may be absent.

Regurgitation Jaundice: The diagnostic problems connected with this group are so difficult for solution, that it may be wise to consider them more in detail. Let us suppose a patient's symptoms began with severe, or colicky upper right quadrant pain, which within a matter of hours or of one or two days was followed by jaundice. One must decide whether the condition is caused by a stone in the common duct or in part protruding beyond the opening of the cystic duct, or by a non-calculous cholecystitis with secondary hepatitis. The jaundice due to stone in the common duct usually becomes more intense and persists. The stools become acholic or nearly so and the urine is found to be dark and to contain bile, although its content of urobilinogen is related to the permanence of the obstruction.

Another factor which further complicates the picture is that pain is commonly associated with carcinoma of the head of the pancreas

and to a lesser degree with hepatitis. Where these uncertainties exist, it is helpful to use other methods of investigation, which are described below.

Gallstones or non-calculous cholecystitis may be the focus from which a cholangeitis or pylephlebitis or hepatitis originates. The jaundice associated with gallbladder colic is usually slight and of short duration, but there are times when a biphasic or indirect reaction may be noted for long periods of time. Increased amounts of urobilinogen may be found in the urine. The history and physical examination in these cases may or may not be suggestive of gallbladder disease; consequently, it is not only justifiable but good practice to do gallbladder dye cholecystography, preferably during periods when the patient is not jaundiced. If this does not give a clear indication of the situation, macroscopic and microscopic studies of the A, B, and C bile frequently throw light on the problem. In patients with intact biliary vesicles, if normal amounts and concentrations of A, B, and C bile are obtained, it may be reasonably assumed that the gallbladder is concentrating bile. If crystals of cholesterol, calcium bilirubinate, bilirubin or calcium carbonate are found, the question becomes more complicated. That overemphasis on the importance of the presence of these materials in the bile has resulted in the removal of a considerable number of normal or medically curable gallbladders, is undeniable. Certainly in this matter the author has been no outstanding exception. As in all cases of this type, experience is necessary for judgment and appreciation of the significance of the findings.

One of the most helpful points is the repeated absence of B bile following successful drainage. This negative finding in association with an x-ray diagnosis of the non-filling gallbladder is strong evidence that the organ is diseased. Again, if there is evidence of only slight or of no concentration of bile in association with the presence of unusual amounts of one or more types of crystals enumerated above, one may reasonably assume that cholelithiasis is present.

At times jaundice is secondary to malignancy of the gastro-intestinal tract or to pressure from duodenal diverticula. An x-ray study should clear up this point.

As a contrast, we find patients whose jaundice has come on insidi-

ously, at times preceded for two or three days by nausea or vomiting with malaise and general irritability. These people, as a rule, are suffering from a catarrhal or infectious jaundice. It is not uncommon to find in them an enlarged spleen, a condition which is unusual except in the later stages of biliary obstruction. The diagnostic problem would be simple except for the fact that hepatitis of toxic origin, biliary obstruction due to a silent stone, or a carcinoma of the head of the pancreas may produce the same predominant symptoms.

SELECTION OF TESTS

Galactose Test (12): It is generally reported that in the early stage of jaundice the use of the galactose tolerance test is of value in differential diagnosis (13, 14, 15, 16). In cases of biliary obstruction, before marked hepatic changes have taken place, the liver is able to metabolize the galactose and only small amounts are excreted in the urine. In patients with hepatitis—infectious or toxic—there is a reduction in the liver's ability to handle this hexose, and increased amounts of it are found in the urine. The test, however, is not without its limitations, and the findings must be accepted with caution. After an obstruction has persisted for a sufficient period of time, increasing numbers of the liver cells become necrotic and sooner or later the test will demonstrate an increased amount of urinary galactose.

Cholesterol: Sufficient carefully done work (17, 18, 19) has now been reported to make the estimation of serum cholesterol a useful undertaking. In cases of jaundice caused by obstruction there is usually an increase of cholesterol, and in cases of hepatitis, a diminution; the amount of the fall apparently being somewhat proportional to the severity of the lesion. In both types, the investigators say, there is generally an increase in the cholesterol-free: cholesterol-ester ratio. Consequently, there is but little if any advantage in carrying out both procedures, inasmuch as a very low cholesterol-ester level usually means a very ill patient—a condition which one should readily recognize clinically.

Bilirubin:

Van den Bergh: To do estimations of bilirubin when it is evident that the patient is jaundiced would seem absurd except for two reasons:

1. A considerable degree of jaundice may be present and be due to

retention alone. It is of fundamental importance to know this, and it may be determined from a qualitative Van den Bergh test.

2. Although it is impossible to correlate the concentration of bilirubin with the severity of the disease, it is true that very high estimations are more apt to be due to hepatitis than to simple obstruction of the bile ducts, if not of long duration.

Icterus Index (20): This test was originated by Meulengracht and its description may be found in any laboratory textbook. Its technic is simple, as one merely matches the color of the serum against varying intensities of potassium bichromate. Many writers consider its quantitative estimations of bilirubinemia more accurate than those made with the Van den Bergh technic. This accuracy is not of great practical use, since only the highest readings by both methods are of any diagnostic import.

Urobilinogen: Its constant absence from the urine in cases of jaundice signifies that no bile is reaching the intestine. It is found in the urine in increased amounts in association with liver damage and when bile reaches the intestine. When it is found periodically in large or small amounts, it is evident that some bile is reaching the intestine or, as happens rarely, that the bile retained above an obstruction has become infected and that the bilirubin is being reduced to urobilinogen. This may be true of infectious or toxic hepatitis, or common-duct stone. The author has seen one case in which a duodenal diverticulum caused periodic obstruction, and in which urobilinogen was at times absent from the urine.

The proper analysis of these laboratory findings and adjudgment of their relationship to the clinical picture will often be sufficient for diagnosis. Another difficulty lies in the differentiation of a tightly fastened common duct stone due to carcinoma of the head of the pancreas.

Courvoisier's law, in my experience, has not been of practical value. The major objections are:

1. One can never be sure that the mass one is palpating is really an enlarged gallbladder. It is practically impossible to distinguish it from an overlying lappet of the liver.
2. At operation it is not uncommon to find an enlarged gallbladder associated with a common-duct stone.

Pancreatic Enzymes: In the presence of jaundice caused by a carcinoma of the head of the pancreas, its enzymes will not reach the duodenum, unless such very rare abnormalities are present, such as a secondary pancreatic duct or a position of the carcinoma which does not completely obstruct the duct. The presence or absence of these enzymes may be determined on the duodenal juice obtained after magnesium sulphate instillation through a duodenal tube which by fluoroscope has been properly directed so that the tip lies below the ampulla of Vater (21).

In the differentiation between stone and cancer this finding is of great value. Unless the stone occludes the ampulla of Vater, pancreatic enzymes should be demonstrable. If they are not, there is either a carcinoma of the head of the pancreas or a stone in the ampulla of Vater, thereby causing occlusion of both common and pancreatic ducts.

Except for the specific purpose of the demonstration and quantification of pancreatic enzymes, the passage of a duodenal tube in jaundiced patients is of questionable value, whether it is done for the purpose of determining the presence of bile in the intestine or for therapeutic reasons. In the first instance, if bile is being passed into the intestine, it can be recognized by examining the urine and stool daily for urobilinogen or urobilin. This is a much better method than placing reliance on a flow of bile into the intestine at the time of the drainage. In the second place it is unproved and unlikely that any hypothetical improvement which might be obtained by non-surgical biliary drainage cannot be equally well gained by the oral administration of drugs. Furthermore, it is an exhausting process for an already tired patient.

Miscellaneous: When jaundice is of the regurgitant type and of sufficient intensity to be visible, it is very doubtful if the data obtained from dye retention tests, quantitative blood bilirubin tests, glucose tolerance tests, estimation of urinary bile acids will add any further information. The more recently proposed tests such as estimation of urinary tyrosine (22) and hippuric acid (23) have not yet been sufficiently tried out to permit the drawing of any deductions. The former would seem to offer scant information, if any, beyond that obtainable by other means.

TREATMENT

Retention Jaundice: With the exception of hemolytic jaundice, the treatment of the diseases in which there is retention jaundice is a medical problem, whereas this rare but interesting disease responds to but one form of therapy—surgery. Liver extract has proved unavailing, and whole blood transfusions are superfluous, in view of the rapidity with which the blood count increases to normal after operation. Before advising a splenectomy, one must be convinced that the patient has hemolytic jaundice, since operation in cases showing some similarity to it has proved disadvantageous (24).

There seems to be no rationale for instituting special treatment in individuals suffering from an unexplained indirect hyperbilirubinemia. They should be watched and it would seem wise to have them refrain from excessive amounts of alcohol and protein. Their management is, in reality, a problem for their individual medical advisor. The fact that no organic basis for their hyperbilirubinemia is demonstrable is but poor reason for believing it does not exist. An indirect Van den Bergh reaction does not indicate an undamaged liver; it only shows that there is no rupture of bile canaliculi.

Portal cirrhosis is frequently associated with an indirect hyperbilirubinemia. There are times apparently during exacerbations of liver cell destruction when a direct reaction is found. Its treatment is mentioned under toxic hepatitis.

Regurgitation Jaundice: It is now established that the liver's store of glycogen is one of its best defensive and curative weapons. Consequently, during periods when the liver is diseased or subjected to damage (from ether, alcohol etc.) efforts should be made to increase the supply of glycogen. This may be effected by giving glucose, saccharose or starch, by mouth when possible—if not, then glucose should be given intravenously.

In cases of jaundice induced by arsphenamine an interesting therapeutic point arises. Craven (30) has shown that the livers of dogs fed on a high fat diet for some weeks before they were given arsphenamine showed less necrosis than the livers of those kept on a high carbohydrate regime before institution of arsphenamine injections. This finding offers, to those who treat syphilis, a fertile field for the investigation of the same problem in man, and might help to settle

the prophylaxis of arsphenamine jaundice; it would leave unsettled, however, the treatment of the established condition. Arguing by analogy from the observation made on dogs, it might be that if these sufferers from toxic hepatitis have arsenic still present in their livers, further activity of the metal could be prevented by putting them on a high fat diet.

We are, however, met with an actual condition not a possibility. Many of these patients have a hypoglycemia and unquestionably should be treated with glucose. We do not know how many of the other less sick patients may develop a hypoglycemia and it would seem probable that these patients should also be treated with glucose in spite of the theoretical possibility that the association of glucose and residual arsenic might further injure an already damaged organ. It seems unlikely that in the arsenic-poisoned liver the residual metal is as important as the need of the organ for glycogen.

The liver presumably has a great deal to do with the synthesis of serum proteins (25). Consequently, it might seem logical to treat patients suffering from severe hepatic damage with protein replacements. However, when Mann treated dogs which had been jaundiced for more than three or more months with a high meat diet, he found that life was shortened and that ascites was likely to occur. By changing to a diet high in carbohydrate and low in meat protein, the ascites was dissipated and a marked improvement was produced in the clinical picture. It is through the de-aminizing action of the liver that the body is able to use the products of protein metabolism, but when the liver is severely damaged, it is only logical that this process should be limited. This state would seem significant enough to make one refrain from overburdening the organ.

The absorption of fats is intimately related to their admixture with bile acids. In the complete absence of these acids the feeding of fats adds very little in the nourishment of the body; hence they should be curtailed unless bile acids be administered. When patients have been operated upon and a biliary fistula has been made, it is easy to collect the bile and instill it into the patient's small bowel through a duodenal tube. The change that takes place in patients, particularly in those who have been jaundiced for some time, is frequently gratifying. In the presence of partial obstruction or in cases of hepatitis

in which bile is reaching the intestinal tract, fats may be added to the diet in normal amounts, since a small amount of bile will emulsify a large amount of fatty acid.

There is no doubt that some jaundiced patients have a markedly increased tendency to bleed. A notable amount of work has been done on the subject, but as yet the question is unsolved. Equally difficult of solution is the problem of picking out the patients who have this tendency to bleed. Many of them will react normally to the tests designated to demonstrate a bleeding tendency, but after operations will behave in a contrary and disappointing manner. It may be that the recent test of Ivy (26) can be utilized to designate these patients, but at present it is too early to be sure.

Where time permits, all deeply jaundiced patients who are to be operated upon should be treated not only as having low liver glycogen stores, but also as potential bleeders. For the latter condition the prophylactic usage of whole blood transfusions (27) and calcium gluconate, intravenously, should be utilized. At present the evidence for using calcium gluconate is not well established. It is my opinion that it is helpful and that some patients have been definitely helped by it, especially those suffering from toxic hepatitis. It is wise to continue such therapy after operation.

Catarrhal or Infectious Jaundice: In Weil's disease, yellow fever and congenital syphilis, we recognize a definite or fairly well classified infectious agent. Catarrhal jaundice, on the other hand, may possibly include a number of etiological factors. The preponderance of evidence points to an infectious basis; whether there is a specific organism or whether there are a number of them is unknown. In only one of the diseases noted above is specific therapy possible, consequently in the others we must rely on sustaining and upbuilding methods. Rest, quiet and a properly selected diet in which there is high carbohydrate are fundamental. Pain is often noted and to allay it such remedial agents as acetylsalicylic acid, codein, and preparin are efficacious. Cinchophen is not to be used.

The use of cholagogues is of questionable value, as they may overtax cells already damaged. If the condition persists for a long time and the appetite fails, the use of bile acids might be considered in spite of their cholagogue action, since they aid in the digestion of fats.

The continued use of laxatives and, by the same token, of biliary drainage through duodenal tube is to be deplored.

It is difficult to see how emptying a gallbladder medically can possibly help a hepatitis. Supposing this measure does stimulate the liver cells to secrete,—and this is doubtful—its use would be as questionable as that of cholagogues. If there are those who believe that jaundice may be caused by a mucous plug obstructing the ampulla of Vater, and who believe that non-surgical biliary drainage is indicated, it may be said that no one has as yet proved that the oral administration of fat or magnesium sulphate is any less efficient in releasing this circular muscle. In the same sense if common-duct stones can be aided in passage by medical treatment, let it be done by the routine methods of oral administration.

Biliary Cirrhosis: The treatment of this condition is surgical.

Toxic Jaundice: One method of diagnosing this condition is by knowledge gained from the patient or by examination of the drugs he has taken. Obviously the first essential of treatment is the removal of the offending chemical. Beyond this the patient should be handled in exactly the same manner as one suffering from catarrhal jaundice. Many of these patients are quite sick and in spite of most faithful and persistent care their livers become more and more damaged until they disappear under the costal margin, the jaundice becomes more intense, and the stool becomes and remains acholic. Nevertheless, intravenous glucose, calcium gluconate and whole-blood transfusions should be continued. In the author's experience an occasional very sick individual improved and it seemed that the therapy had something to do with it.

It may be that cases of *portal cirrhosis* should come under this classification. Eliminate from the diet and fluid intake toxic materials, particularly high proteins and alcohol. The rôle of tobacco is unknown and it may have some detrimental action; the extent of its use should be considered by the physician. To routinely forbid its use seems completely unjustifiable and is too hard on the patient who has so little time and so few pleasures left.

Common Duct Stone: For this condition there is only one form of treatment—surgery.

The medical man must interest himself to see that the patient has the proper pre- and post-operative care. This will usually be a mere

matter of looking on, since the well trained surgeon is completely cognizant of the need for care in this condition. If possible, the operation should be held off a few days until the patient's liver has been stored with glycogen, and if the jaundice is of long standing, until calcium gluconate and whole-blood transfusions have been given as a possible prophylaxis against bleeding. As stated before, this treatment should be continued post-operatively.

If it is the judgment of the surgeon to institute long continued external biliary drainage, it is well to collect the fluid under sterile conditions and return it to the patient through the duodenal tube.

Cholelithiasis and Cholecystitis with Jaundice: The diagnosis and treatment of these conditions have been described fully elsewhere (28). In brief it may be stated that when stones in the gallbladder are associated with pain and jaundice there is only one form of therapy—surgery. If they are not giving symptoms, but are nevertheless associated with attacks of jaundice, again surgery is indicated.

Cholecystitis, when associated with jaundice, should be regarded as a very likely focus from which the hepatitis has originated and for this reason a cholecystectomy should be performed. In this type of case it is very unlikely that any permanent improvement will be obtained by medical drainage through the duodenal tube. When surgery is indicated and the evidence of hepatic drainage is marked, the same careful attention to pre-operative care described above should be utilized.

Carcinoma of the Head of the Pancreas: Unless the disease has progressed too far, or the condition of the patient is unsatisfactory, surgery offers a temporary but appreciable state of well being. The operation consists in making an opening between the gallbladder and some portion of the upper intestinal tract, the stomach, duodenum or jejunum. Just what is the operation of choice is as yet undecided. If a cholecystogastrostomy can be made so that food contents are not forced into the gallbladder, this would seem the best location for attack. However, opinion is undecided, and the decision must be left to the judgment of the surgeon. The same pre- and post-operative care must be taken as in calculous obstruction of the common duct. If the operative technic is satisfactory and infection does not follow, the results are very gratifying.

Miscellaneous Conditions: They occur rarely and such medical or

surgical treatment as the particular symptoms require should be carried out in accordance with the therapy outlined above.

SUMMARY

In the above memorandum I have endeavored to give a résumé of the normal physiology of biliary excretion. More detailed descriptions and references may be found in the articles of Rich (3), Mann (25), Barron (5), and the writer (29). Only by a thorough knowledge of the subject is it possible to gain an understanding of the various causes of jaundice.

With jaundice established it is possible, with the aid of history, physical examination and various tests, to locate the source of the mischief in a large percentage of patients. The diagnosis once made, therapy, be it specific, supporting, or surgical, may then be instituted.

There will be a certain number of cases in which the diagnosis will remain unsolved. In these the most important decision will lie between the choice of medical or surgical treatment. To lay down any hard and fast rules for choice of procedure is impossible. However, certain broad principals may be suggested.

In youth and early adult life the liver pathology is generally a hepatitis for which surgery affords no aid. In later life the importance of the consideration of surgery is paramount. A commonduct stone, if permitted to obstruct for too long a period, will cause destruction of liver cells. In cases of carcinoma of the head of the pancreas, surgery is not only palliative but of value in prolonging and affording a comfortable existence. Beyond middle age when the diagnosis lies between some obstructive condition and a hepatitis (generally infectious), surgery is indicated after an adequate period of observation. Lives may be saved, and if the condition turns out to be one of a medical type, little damage has been done, particularly if there has been proper pre-operative care and the choice of anesthetics has been limited to those least toxic to the liver.

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PROGRESSIVE EXOPHTHALMOS FOLLOWING THYROIDECTOMY[1]

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The four cardinal signs of hyperthyroidism are goiter, exophthalmos, tremor and tachycardia. With the exception of goiter, we lack an explanation of any of these phenomena. In this disease the thyroid gland is excessively active and surgeons have demonstrated that its partial removal eliminates or interrupts most of the symptoms. Recently, the relationship of the pituitary gland to hyperthyroidism has been suggested by the demonstration of a substance present in the anterior lobe of the pituitary gland which has the power to stimulate thyroid activity (thyrotropic hormone), but there are still those who cling to the indefinite hypothesis that the primary cause of the disease lies in the sympathetic nervous system which, being somewhat overactive itself, overstimulates the thyroid gland.

Exophthalmos usually accompanies and parallels hyperthyroidism, but in some cases it is entirely absent. Rarely it involves one eye and spares the other. After adequate surgical treatment of the thyroid gland the exophthalmos usually abates or disappears, but there are some cases in which the condition of the eyes remains unchanged. Finally, there is a small and interesting group of cases which present progressively increasing exophthalmos even after thyroidectomy has brought about relief of all the other symptoms such as tachycardia, tremor, nervousness and increased rate of metabolism. Sixteen such cases have been collected from the records of the Wilmer Ophthalmological Clinic of the Johns Hopkins Hospital during the past ten years. A study of these cases is undertaken in the hope that some light may be thrown on the mechanism of the exophthalmos which, although a part of the clinical picture of hyperthyroidism, would seem

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to be caused by some factor outside of the thyroid gland itself. The main ideas advanced to explain the exophthalmos of Graves' Disease are:

1. An increase in the orbital fat.
2. Oedema of the normal orbital contents.
3. Congestion of the vascular bed of the orbit.
4. Relaxation of the extra-ocular eye muscles.
5. Contraction of the smooth muscles of Müller, Landström and Hesser.
6. Hypertrophic myositis of the extra-ocular muscles.

There are still advocates of the early theory, advanced by Basedow (1) and many others, that the eye is forced forward by an increased amount of orbital fat. It is difficult to believe that, in a disease characterized by loss of body weight, an inert substance such as fat should be deposited and grow in the orbit with such vigor that it could raise the intra-orbital pressure sufficiently to push forward a normally resisting eye. It is possible that once the eye is protruded the increased orbital space might be filled by additional adipose tissue.

Several patients with post-thyroid exophthalmos have been subjected to exploratory operations on the orbit, to exclude the possibility of retrobulbar tumors. In such patients the common finding is that Tenon's capsule is displaced anteriorly by hypertrophied or oedematous normal orbital contents. Oedema of the normal orbital contents must be considered a result of some more profound disturbance, rather than as a primary affair. It has been suggested that this oedema is due to venous stasis, but a more logical explanation is that the oedema (or hypertrophy) is secondary to some chronic inflammatory reaction.

Congestion of the vascular bed of the orbit has been assumed on the basis of vasodilatation through the sympathetic system or toxic action directly on the vessels themselves, but has been largely rejected because the eye neither pulsates nor is itself unduly congested in every case. A suggested explanation for this hypothetical venous congestion is constriction of the emissary veins by the contraction of the involuntary muscles of the orbit, especially Müller's muscle, but this is highly improbable when the abundant collateral circulation is considered.

Loss of tone of the extra-ocular muscles has been advanced to explain proptosis (Parker, 1921) (2), and a case has been described in the Vanderbilt Hospital in which the eye moved forward as the eyelids were opened and receded when the eyelids were closed. Theoretically, it is difficult to eliminate this as a contributing factor in the production of exophthalmos in a disease known to exhibit weakness of striated muscle throughout the body. However, the actual position of the eye in the orbit is not wholly dependent on the extra-ocular muscles. Whithall's (3) description that . . . "the eyeball is delicately poised in fascia and fat of the orbit. . . . A state of equilibrium is maintained by the tonicity of all the ocular muscles. . . . As a whole, the four recti may be regarded as retractors of the eyeball; they are antagonized by the two obliques, which from their origins and directions are protractors and this is probably the active element in maintaining the equilibrium of the globe" is probably correct. Loss of tone of the ocular muscles would leave the globe *in statu quo*. Loss of tone, however, may remove a restraining influence and thus allow some new and otherwise unimportant force to push the eye outward.

The great majority of writers look to the smooth muscle of Müller (4) and Landström (5) and Hesser (6) for the explanation. These muscles attain reasonable proportions in many laboratory animals, and in these animals stimulation of the cervical sympathetic nerves is followed by visible protrusion of the eye. MacCallum (7) reported such experiments in 1904 and described in dogs a cone-shaped muscle around the posterior half of the bulb, which contracted in response to stimulation of the sympathetic nerve. The smooth muscle described by Müller is rudimentary in man and that of Landström and Hesser forms a thin sheath connected with Tenon's capsule around the anterior half of the bulb and is visible only in microscopic sections. It is probably innervated by the cervical sympathetic chain. Stimulation of the cervical sympathetic nerves in man fails to produce protrusion of the eye. Animals which show a protrusion of the eye after stimulation of the cervical sympathetic still show the same phenomenon after resection of Landström's muscle, although they do not if the smooth muscle back of the eye is cut (MacCallum). Friedgood (8) in 1930 reported studies on exophthalmos encountered in association with hypertension, and while agreeing with Barker

and Hanes (9) (1909) that the two conditions were probably produced by a common cause, he accepted as proved that exophthalmos in man is the result of hyperactivity of the sympathetic nervous system. However, hyperactivity of the sympathetic nervous system in patients with post-thyroid exophthalmos is little more than a figure of speech. In support of this skepticism, it may be pointed out that exophthalmos is never extreme in cases of hypertension and also that hypertension does not occur in uncomplicated cases of hyperthyroidism. Friedgood's paper provided the material for an editorial in the Journal of the American Medical Association, June 30, 1934 (10), which considers the subject of the mechanism of exophthalmos in hyperthyroidism as settled. We greatly fear it is not so simple.

Naffziger (1931–32–33) (11) has reported four cases of progressive exophthalmos following thyroidectomy in all of which he found the extra-ocular muscles enlarged from three to eight times their normal size, and the site of inflammatory lesions with degeneration of the muscles, fibrosis and cellular infiltration. He believes this hypertrophic myositis is responsible for this type of progressive exophthalmos and advises intracranial decompression of the orbit and opening of the ring of Zinn as a curative procedure.[2] Other reports have been made of idiopathic myositis of the orbit. Askanazy (13) (1898) studied in four cases of exophthalmic goiter striated muscles, including the orbital muscles, and found them all with the exception of the heart muscle subject to fatty degenerative changes. The muscle of the diaphragm frequently showed the most advanced changes. Gasteiger (14) (1932) reported a case clinically similar to those of Naffziger in which he thought the exophthalmos was produced by myxoedematous swelling of the orbital contents. The exophthalmos decreased under replacement thyroid therapy. In Naffziger's four cases and two others examined by him, the post-operative basal metabolic rate varied from normal to −32. All of these patients showed puffiness of the lids, scleral oedema and lacrimation. The mobility was impaired in five of the six and swelling of the optic disc with impaired vision was present in five.

[2] Dollinger (12) in 1911 reported one case of progressive exophthalmos following goiter in which he decompressed the orbit by removing a portion of the temporal bone which forms the outer wall of the orbit. This would seem to offer a safer approach than the intracranial one advised by Naffziger.

We have recently examined sections of the eye muscles of three patients exhibiting post-thyroid exophthalmos. In one of these biopsy of the extra-ocular muscle showed changes similar to those described by Naffziger, while biopsy in the two other cases showed normal muscles. Three years ago Friedenwald (15) examined all the eye muscle material available from cases of hyperthyroidism in the Department of Pathology of the Johns Hopkins Hospital. This material is disappointingly scarce, since comparatively few patients die from hyperthyroidism and even if postmortem examination is allowed, permission to examine the eyes is usually denied. Of the six such cases he examined, the extra-ocular muscles were normal in five and in one the muscles were slightly enlarged and showed degenerative changes and cellular infiltration. Photo-micrographs of sections from eye muscles of two cases presenting these changes are shown in Figs. I, II, III, IV. Of particular interest in these sections is the presence of islands of round cells suggesting lymphocytic germinal centers very similar to those encountered in the thyroid gland in hyperthyroidism. What they signify is not clear, but Rich (16) also has recently pointed out similar lesions in the diaphragmatic muscle of a patient dying from Addison's disease with atrophy of the adrenal cortex, and in this case the thyroid gland was also particularly rich in these germinal centers (Figs. V and VI).

The fifteen cases which constitute our series of progressive exophthalmos following thyroidectomy are all more or less alike. Eleven of the patients were males and four were females. This is interesting in view of the preponderance of women over men with hyperthyroidism. The average age was thirty-nine years. The youngest was twenty-four years, and the oldest sixty years. In every case thyroidectomy relieved all the general symptoms except the exophthalmos. If an exophthalmos was present in these patients before thyroidectomy, it was always of low degree and receded somewhat after thyroidectomy, only to recur and gradually grow worse. The secondary protrusion of the bulbs began anywhere from ten days to two years after operation. Paralysis, partial or complete, of certain extra-ocular muscles, usually the superior rectus, occurred in seven cases. The postoperative basal metabolic rate was within normal limits in all but three patients, two of whom had postoperative myxoedema with basal metabolic rates of -30 and -35, respectively,

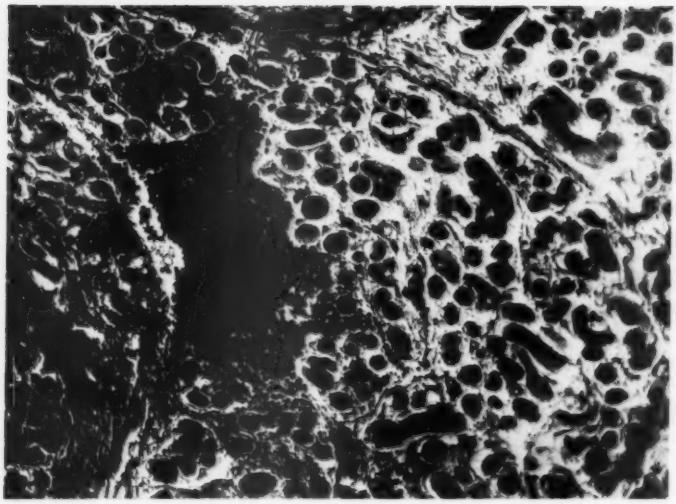

FIG. I. PHOTOMICROGRAPH OF THE EXTERNAL RECTUS, SHOWING AN ISLAND OF ROUND CELLS; LOW POWER

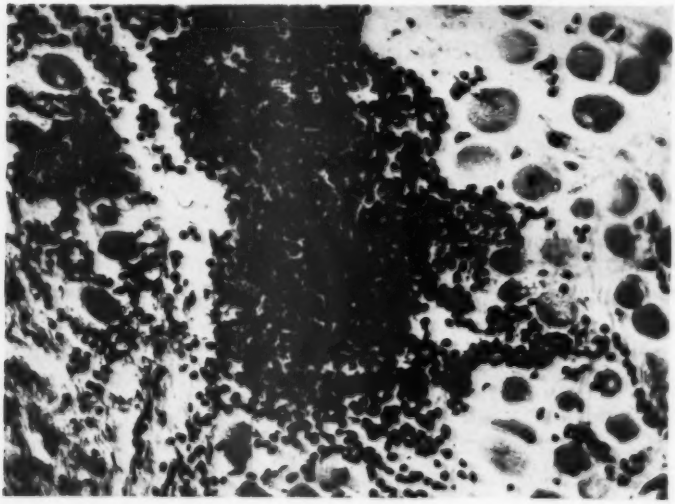

FIG. II. PHOTOMICROGRAPH OF THE EXTERNAL RECTUS, SHOWING AN ISLAND OF ROUND CELLS; MEDIUM POWER

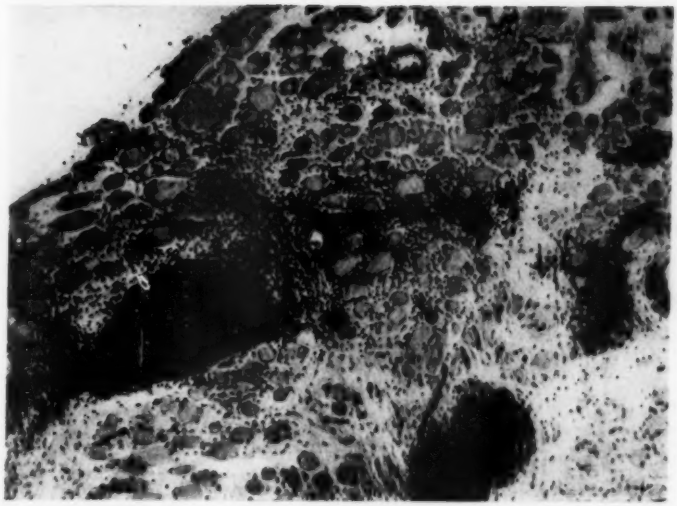

FIG. III. PHOTOMICROGRAPH OF THE EXTERNAL OCULAR MUSCLE FROM THE CASE REPORTED BY DR. BURCH, SHOWING SIMILAR COLLECTIONS OF LYMPHOCYTES; LOW POWER

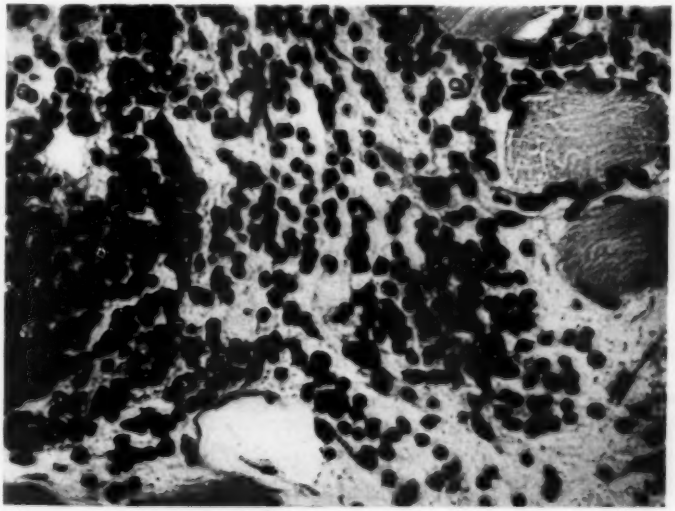

FIG. IV. PHOTOMICROGRAPH OF THE EXTERNAL OCULAR MUSCLE FROM THE CASE REPORTED BY DR. BURCH, SHOWING SIMILAR COLLECTIONS OF LYMPHOCYTES; HIGH POWER

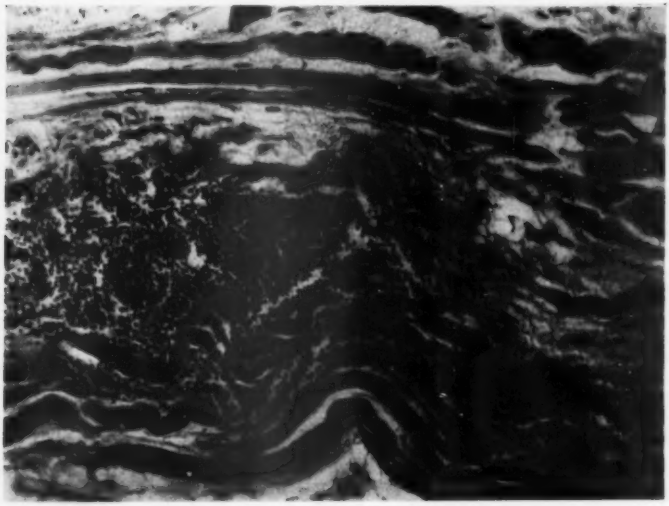

FIG. V. PHOTOMICROGRAPH OF A SECTION OF THE DIAPHRAGM FROM CASE DESCRIBED BY DR. RICH; LOW POWER

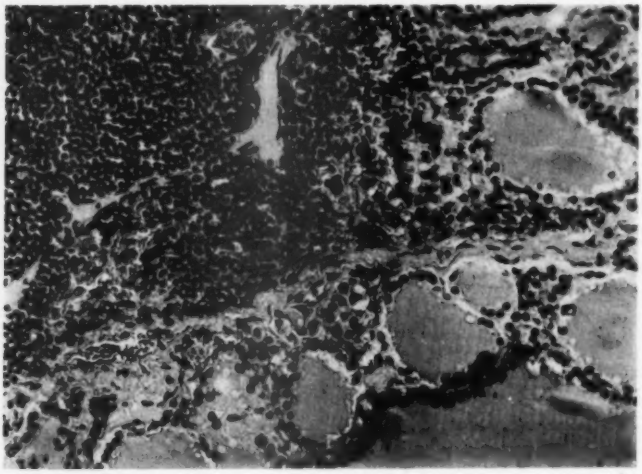

FIG. VI. PHOTOMICROGRAPH OF THE THYROID GLAND FROM CASE DESCRIBED BY DR. RICH; MEDIUM POWER

while the third had a basal metabolic rate of +36. Thyroid extract was given to five patients of this series, with no effect on the exophthalmos.[3] Lugol's Solution was likewise without effect in two other cases. Five were mild cases, which have as yet required no specific treatment. In three patients the secondary exophthalmos was so intense that it was necessary to partially suture the external palpebral fissure (lateral tarsorraphy) to give protection to the cornea. In one of these patients the exophthalmos has continued to progress. X-ray treatment is being given to the orbits with the hope of reducing the swelling. While some improvement has been noted, it is still too soon to make any definite statement on the value of radiation in this patient. Two other severe cases have shown spontaneous improvement over the course of years without specific treatment.

In one patient in this series the exophthalmos was so fulminating that necrosis of the cornea with loss of vision occurred and bilateral enucleation of the eye was performed in another clinic seven months after the thyroid operation. Following enucleation, the orbital contents continued to hypertrophy and finally bulged out between the eyelids. In this condition, two years later, she was admitted to the Johns Hopkins Hospital, where a plastic operation was performed to remove the deformity. Pathological examination of the excised mass showed no tumor cells, only normal connective tissue and fat. No muscles were identified, but in the redundant conjunctiva there was a definite inflammatory reaction with round-cell infiltration. The progressive hypertrophy of the orbital contents after enucleation of the eyes strongly indicates that the same process was present before enucleation and was reponsible for the exophthalmos. A similar case was described by Griffith (19) in 1898.

In one patient of this series (Fig. VII) a modified Naffziger operation was performed by Dr. Walter Dandy, with intracranial removal of the orbital plate, but without opening the optic foramen. In

[3] Zimmermann (17) in 1929 reported eight similar cases, in four of which there were symptoms of hypothyroidism and in three others basal metabolic rates of slightly less than −15%, and Des Brisay (18), in 1934, added three such cases, in two of which there were low basal metabolic rates. On the other hand, we have under observation at present a patient with progressive postoperative exophthalmos whose basal metabolic rate is +36%.

this patient there was marked bilateral choked disc (Fig. VIII) and vision was reduced to 20/70 in the right eye and 20/200 in the left eye. After the orbital decompression the papilloedema subsided (Fig. IX), the vision improved to 20/30 plus 4 in the right eye and 20/20 minus 2 in the left eye, and the exophthalmos receded. Ex-

FIG. VII. PHOTOGRAPH OF PATIENT BEFORE ORBITAL DECOMPRESSION

amination of the specimen of extra-ocular muscle removed at operation showed degenerative and infiltrative lesions similar to those described by Naffziger (Fig. II). Dandy performed a similar operation on another patient, not included in this series, in whom secondary exophthalmos developed during a recurrence of hyperthyroidism and persisted in an extreme degree, causing her to seek relief. In this

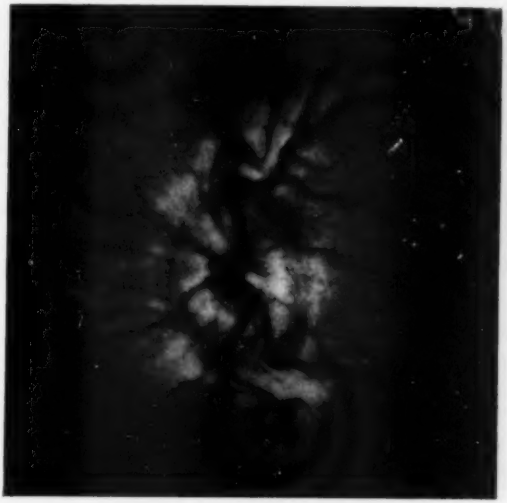

FIG. VIII

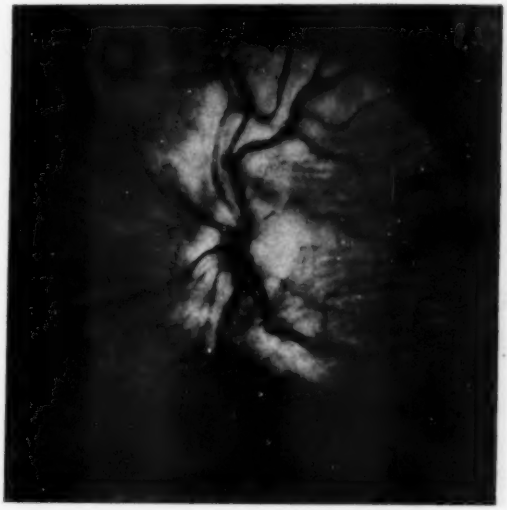

FIG. IX

FIGS. VIII AND IX. PHOTOGRAPH OF EYEGROUNDS BEFORE ORBITAL DECOMPRESSION, SHOWING CHOKED DISC AND AFTER, SHOWING NORMAL DISC.

patient the orbital decompression resulted in lessening the exophthalmos on the right side, while there was little effect on the left eye. Examination of the sections of eye muscle from this patient taken at each operation revealed normal muscle. In this patient, however, the exophthalmos was stationary and not progressive.

In two patients of this series, the orbits were explored by the Krönlein route in view of the possibility of retrobulbar or orbital tumors. In both of these patients there appeared to be more than the normal resistance of the orbital contents. Tenon's capsule appeared displaced anteriorly and the orbital contents were normal but oedematous or hypertrophied. In one patient a small piece of the external rectus muscle was removed for biopsy, but the histological picture was that of a normal extra-ocular muscle.

COMMENT

Observations made at operation on five of the cases here reported throw some light on the mechanism of exophthalmos. In two cases the orbit was explored, in two it was decompressed from above, and in one, in which the eyes had been previously removed, an exploratory and plastic operation was performed. In one of these patients, on whom a Krönlein operation was done, the periosteum containing the orbital contents was under definite tension, so that, when it was opened, the orbital fat extruded. This was true also in one of the patients decompressed from above. The orbital fat was not greatly increased in amount as far as could be determined in these or in any of the other three patients operated upon.

In three cases presenting progressive exophthalmos, tissue from the extra-ocular muscles, obtained either by exploratory operation or postmortem examination, showed the characteristic inflammatory reaction associated with large islands of round cells. The striking similarity of this reaction to that noted in the thyroid gland during hyperthyroidism and also in a case of Addison's Disease brings up the possibility that it represents a hitherto unnoticed generalized lymphoid response to some form of endocrinopathy. If such is the case, this reaction occurring in the orbit might well have been responsible for the swelling of the orbital contents with resulting increase in orbital pressure and exophthalmos. Dr. Burch (20) has been kind

enough to send us sections of the eye muscles from the case he reported in 1929 and they show beautifully the islands of round cells previously described (Figs. III and IV). In 1934 Zalka (21) (Budapest), stimulated by the work of Naffziger, reported a careful study of the extra-ocular eye muscles in sixteen patients dying with Basedow's Disease. He was impressed by the increase in size of the muscles and also by a pronounced interstitial infiltration, which was found in almost all cases and consisted of large and small foci of lymphocytes. These also occurred primarily in the interstices of the straight ocular muscles. He considered this change to be of an inflammatory nature and rejected the idea of Schütz (1923), who had suggested that it might be part of the phenomenon of status lymphaticus. Zalka concluded that changes in the ocular muscles were the result of the exophthalmos and increased function, rather than the cause of the exophthalmos as suggested by Naffziger. However, the type of case in which the orbital contents continue to enlarge and extend from the orbit even after enucleation of the eye demonstrates that some active force producing swelling of the tissues is in operation.

Marine (22) was able to produce exophthalmos in laboratory animals by injection of thyrotropic hormone of the anterior lobe of the pituitary gland. This occurred more rapidly in guinea-pigs from which the thyroid had been removed than in normal guinea-pigs. He believed this phenomenon is produced by stimulation of the higher sympathetic center in the hypothalamus with resultant over-activity of the cervical sympathetic and contraction of the orbital smooth muscle. However, this awaits further proof. He also believed that deficiency of thyroid secretion is important in the production of exophthalmos. Progressive exophthalmos after thyroidectomy, as described in this report, clearly demonstrates that exophthalmos may develop in the absence of clinical hyperthyroidism. On the other hand, hypothyroidism does not appear to be necessary. It is to be remembered that exophthalmos usually accompanies hyperthyroidism and as a rule is diminished by partial removal of the thyroid gland. While exophthalmos may develop in cases of hyperthyroidism after the removal of the thyroid gland when the metabolism is normal or subnormal, it does not occur in patients from whom the thyroid has been totally removed for the relief of heart failure.

If one is to incriminate the thyrotropic hormone, one must postulate some other additional factor which is present in the disease known as hyperthyroidism. Finally, if the thyrotropic hormone plays a rôle in human exophthalmos, we believe that it functions through some mechanism other than over-stimulation of orbital smooth muscle by way of the sympathetic nervous system. Since a widespread characteristic lesion of the striated muscles of the body is known to occur in cases of thyroid disturbance, it seems likely that the collection of round cells is part of the same process. The presence of this lesion in the extra-ocular eye muscles, and the hypertrophied orbital contents, offer a link in the chain of explanation of exophthalmos.

CONCLUSIONS

1. Exophthalmos not infrequently occurs and progresses after adequate sub-total thyroidectomy in cases of hyperthyroidism. Exophthalmos does not follow total ablation of the thyroid gland in cases without hyperthyroidism.

2. Mild cases of post-thyroid exophthalmos tend to recover spontaneously, but in other cases the exophthalmos may be so severe that vision is threatened either by exposure of the cornea or papilloedema and optic nerve changes. In these cases lateral tarsorrhaphy and decompression of the orbit may be indicated.

3. The actual cause of exophthalmos of Graves' Disease is not clear. The association of a characteristic lesion of the striated muscles with hyperthyroidism has been pointed out and its possible rôle as a contributing factor in the production of exophthalmos suggested.

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STUDIES ON THE IMMUNIZING SUBSTANCES IN PNEUMOCOCCI

V. The Effect of Alkalis on the Immunizing Properties of a Type I Pneumococcus Polysaccharide[1]

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Interest in the study of the pneumococcus during the last five years has been exhibited in testing the possibility of isolating from this organism an essential antigen complete in respect to stimulation of active immunity. On the one hand, Heidelberger and Avery (1) had reported their fundamental work in isolating and characterizing the specific polysaccharides of Types I, II, and III pneumococci, and found them to be nonantigenic. Conversely, other investigators, notably Schiemann and Casper (2), Saito and Ulrich (3), Wadsworth and Brown (4), Zozaya and Clark (5), as well as the senior author (6), reported the isolation of substances which were at least largely the specific polysaccharide, and which were shown to be antigenic. In the meantime, Avery and Goebel (7) published an explanation of their earlier failure to show the Type I polysaccharide of Heidelberger and Avery to be antigenic in the fact that during the process of isolation acetyl groups originally present were saponified. Sevag (8), Enders (9), and more recently Heidelberger (10) have confirmed these observations. Our studies, on the other hand, have led us to believe that if there are acetyl groups present in the Type I polysaccharide, they have no significance in respect to the active immunizing property of this substance.

The problem of the discovery of the essential immunizing antigen

[1] This is one of a series of studies carried out in part under a grant from the Influenza Commission of the Metropolitan Life Insurance Company, and in part by the Pneumonia Fund of Johns Hopkins University Medical School.

of the pneumococcus has been greatly simplified by these recent studies of the various investigators. The important step is an agreement among many workers that a polysaccharide of the pneumococcus may be an active immunizing antigen. As a matter of fact, the knowledge that an immunizing dose of an active polysaccharide may be as small as 0.5 c.c. of 10^{-8} dilution or even less renders difficult the proof that any other substance of the pneumococcus cell is antigenic in respect to an active immunity response. For obviously an inert fraction might appear to be antigenic as a result of admixture with a trace of highly active soluble specific substance. Thus, in our study of the relative activity of the different fractions of the pneumococcus (11), consideration was given to the possibility of admixture of inert material with one highly active.

Nevertheless, these possibilities are suggested by the results of the various investigations: First: More than one product of the pneumococcus stimulates active immunity; for example, the alcohol-soluble antigen of Perlzweig and Steffen (12), the cellular polysaccharide of Wadsworth and Brown (4), the acetyl polysaccharide of Avery and Goebel (7), and Enders (9), the toxin of Coca (13), and others; Second: Differences in both chemical nature and biological activity are due to intermediate products of synthesis, for it seems illogical that only the final product, the complete polysaccharide, is present in an actively growing culture. Third: The active immunity response is the result of a single substance of a definite molecular structure but labile in nature and thus readily altered by chemical manipulation. This last consideration was emphasized in the results reported on the "isolation of a non-protein and non-polysaccharide antigen of the pneumococcus" (6), and led us to conduct the experiments given in this report.

The present study is an investigation of a single preparation of a Type I immunizing antigen in relation to some of its chemical characteristics. The material was a purified preparation consisting of a mixture of seven samples of Type I polysaccharide prepared by different methods both from the supernatant broth and from the bacterial cell. It was treated, primarily to remove protein, as follows: The pooled material, 11.5 grams, was dialyzed 24 hours in a thin cellophane tube. The volume was made to a liter, HCl added

to make the concentration N/10 acid, and $NaNO_3$ to make normal concentration of this salt. A precipitate formed and was separated by centrifugation. To the clear supernatant fluid 3 volumes of ethyl alcohol were added to precipitate the polysaccharide. The precipitate was somewhat yellow. It was dissolved at pH 7, the insoluble matter removed by centrifugation, and again HCl was added to the clear neutral solution to make N/10 acid, and finally $NaNO_3$ to make normal salt concentration. This time no precipitate formed. Three volumes of alcohol were added, and the resulting heavy voluminous precipitate was washed with acid alcohol, alcohol and ether, and finally dried. The yield was 9.07 grams. The preparation was definitely yellowish. All well-recognized tests for protein were used on this preparation and found to be negative, including a test for sulfur on a large sample of material.

The investigation includes a chemical and a biological study of the above preparation (A) and of four samples prepared from it in which treatment with alkali was the important variable. Sample B is the original (A) dissolved at pH 7 in a concentration of 1 mg. per c.c., and then made alkaline to N/10 concentration with NaOH and heated in an Arnold sterilizer at 100°C. for 30 minutes. To the alkaline solution, after cooling, were added 2 volumes of a 1:1 alcohol-ether mixture. The white precipitate which formed was washed thoroughly with alcohol, alcohol-ether, and dried. Sample C was made from a portion of the foregoing fraction (B) by treating a neutral aqueous solution, containing 1 mg. per c.c., with one-tenth the volume of concentrated NH_4OH at 4°C. for 18 hours. The NH_4OH solution was precipitated by dilution with 2 volumes of 1:1 alcohol-ether, washed repeatedly with alcohol, alcohol-ether, and dried. Sample D was made from the original material (A) by treatment with NH_4OH as in C. Sample E was prepared from D by the same procedure as B, in other words, heating at 100°C. in the Arnold sterilizer in N/10 NaOH for 30 minutes. These five samples were studied alike as nearly as possible under the same conditions. All samples except the original material were snowy white.

CHEMICAL STUDY

Further work on a "possibly non-protein and non-polysaccharide immunizing antigen" (6) of the pneumococcus led us to believe that

this agent was a modified form of a polysaccharide for the following reasons: The molecule was relatively large (non-dialyzable); the viscosity was almost as great as that of the polysaccharide of Heidelberger and Avery; and hydroxyl groups were present. On the other hand, the Molisch test was negative. This latter fact indicated the probable destruction of aldehyde groups. A similar preliminary study of the preparation described above showed it also to be a modified form of the pneumococcus polysaccharide.

Consequently, chemical analyses were made to ascertain in these fractions treated with alkali, the content of nitrogen, "acetyl" groups, alkyloxyl, optical activity, viscosity, ash, and tests for aldehyde groups, *i.e.*, glucose number, bisulfite and iodine reactions. Nitrogen was determined colorimetrically with a modification of Folin and Denis' micro-Kjeldahl method (14). The "acetyl" was estimated with a method consisting of vacuum distillation from acid, in which glucose gave negative results and pentacetyl glucose yielded theoretical values. The micro-Zeisel procedure (15) was used for estimation of the alkyloxyl content. The samples were analyzed for the glucose number with a modification of the Shaffer, Hartmann and Somogyi copper titration method (16). The usual polariscopic method for optical activity was employed, using a 1 per cent solution of the polysaccharide for analysis. Ash was quantitated with Ball's micromethod (17). The bisulfite addition reaction was set up in duplicate as follows: To each sample containing 10 mgs. of polysaccharide made up to 10^{-3} dilution were added 10 c.c. of 1.2 per cent $NaHSO_3$. The flasks were stoppered tightly and kept at room temperature over night. The excess $NaHSO_3$ was titrated with 0.1 normal iodine solution, 1 per cent starch solution being used as indicator. Control samples of bisulfite and water were also set up under the same conditions. The method for determining the iodine reaction was that reported by Willstätter and Schudel (18). Viscosity was determined with the Ostwald viscosity tube. Freshly distilled water was used for calibration of the pipette and the determinations were carried out in a constant temperature water bath at 30°C. The viscosity of a given sample was recorded as the number of seconds required for its flow through the same vessel.

The results of these tests are given in table 1. The control material on vacuum distillation from acid gave an acetyl equivalent of 7.35

per cent, or three times more than any of the others and seven times more than the least, sample C. The alkyloxyl determination showed a percentage of 2.9 in the control (A), but complete absence in all the other samples. Appreciable differences were shown in optical activity, glucose number, bisulfite and iodine reactions.

The results of this chemical analysis indicate significant alteration in the polysaccharide molecule. The sample treated with NaOH (B) is stripped of its alkyloxyl group, aldehyde group, its ability to react with bisulfite and iodine, as well as being reduced more than 80 per cent in "acetyl" content. Since reducing sugars were absent after hydrolysis and the Molisch test was negative, as well as the

TABLE 1

Chemical study

SAMPLE	NITROGEN	ACETYL (?)	ALKYLOXYL	OPTICAL ROTATION $[\alpha]_D$	VISCOSITY* AT 30°C.	ASH	GLUCOSE NUMBER	BISULFITE ADDITION, 1.2 PER CENT	IODINE REACTION, N/10
	per cent	*per cent*	*per cent*		*seconds*	*per cent*		*cc.*	*cc.*
A. P46 control material	5.20	7.35	2.9	+150°	79.6	10.0	15.6	11.9	1.3
B. (A) treated with NaOH	4.65	1.03	0	0	81.1	6.75	0	0.23	0.03
C. (B) treated with NH_4OH	4.65	0.78	0	+190°	80.4	5.5	0	0.14	1.2
D. (A) treated with NH_4OH	4.2	0.80	0	+170°	83.5	6.55	12.0	5.84	0.2
E. (D) treated with NaOH	4.2	2.0	0	+20°	67.1	8.6	10.25	0.14	0.33

* Distilled water in the same vessel required 63.4 seconds.

bisulfite and iodine reactions at low level, apparently there has been a rearrangement of original material in such a way that union between different units of the polysaccharide is no longer glucosidic. Consequently it is possible that the uncombined aldehyde groups are oxidized or otherwise destroyed by the alkaline treatment, as is the case with glucose under identical conditions. This conclusion in our opinion is justifiable; and yet there is a possibility that a rearrangement may have been made to a trehalose type of union in the polysaccharide and that the bisulfite and iodine reactions are connected with enolization. It is to be emphasized, however, that treatment of a Type I polysaccharide with dilute HNO_3 is the only method so far

found which will produce a modification such that hot NaOH treatment results in the above described effect.

When this NaOH-treated sample was subjected to another alkali, NH_4OH, there was an unexpected alteration in the optical activity, a change from zero rotation in B to $+190°$ in the NH_4OH treated sample C. In other words, there was complete reversal in optical activity from a rotation of $+150°$ in the original sample to zero in the NaOH treated (B) and then to $+190°$ in this NH_4OH treated preparation (C). Peculiarly enough the iodine reaction was restored with NH_4OH treatment. In addition it is to be pointed out that there was a slight decrease in the ash from 6.75 to 5.5 per cent.

In the original material treated with NH_4OH (D), a great reduction of acid on vacuum distillation occurred, complete destruction of alkyloxyl group and the iodine reaction, slight reduction in aldehyde group, 50 per cent in bisulfite addition, and a rise in optical activity. When this sample was treated with NaOH (E), the amount of acid on vacuum distillation was increased (certainly no indication of acetyl, but rather the splitting off of terminal carboxyl radicals), aldehyde reduced slightly, and bisulfite addition as well as optical activity decreased. All of these findings, except splitting off of the alkyloxyl group and the changes in optical activity, indicate alteration of aldehyde groups, probably oxidation. That a reversible alteration in optical activity has occurred is obvious. In the cases of the original material treated with NaOH (B), and this sample further treated with NH_4OH (C), it is possible that we are dealing with an inactive polyhexuronic acid, sample (B), and an active polyhexuronic acid, sample (C). This is at present simply a hypothesis; and it is not to be inferred that it is proven that sample B is a racemic form of the original A, and that C, the active acid, is a stereoisomer of B.

The unexpected results following treatment with NH_4OH were observed in attempts to study possible aldehyde ammonia reactions. However, the results with NH_4OH have been found to be by no means specific. For any of many organic bases, including pyridine, brucine, isoquinoline, ethylamine, methylamine and others, produce a similar effect. The reaction is not a chemical addition (see table 1), but apparently an effect produced by hydroxyl ion. For the use of very

dilute NaOH or KOH, although in the concentrations used not as effective as organic bases, results in a like reaction.

BIOLOGICAL STUDY

The biological study includes (1) estimation of protein precipitated from a dilution of Type I specific immune horse serum containing 120 units per c.c., (2) precipitin titer, (3) active immunity in mice, and (4) the combining equivalent or the number of protective units neutralized by a microgram of each preparation. Each of these tests was carried out on all samples in one day under identical conditions, and was repeated at least twice; in the case of estimation of antigenicity three to six times. The method for determining the amount of antibody protein precipitated was that described by Heidelberger, Sia and Kendall (19). The precipitin titer was estimated by the usual serological technic. Immunity in mice was determined by a single injection of 0.5 c.c. of dilutions varying as follows: 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 4×10^{-8}, 2×10^{-8}, 1.3×10^{-8}, and 10^{-8}. On the seventh day after inoculation the mice were injected with 500 lethal doses of pneumococci, *i.e.*, a dose of 0.5 c.c. of 10^{-6} dilution of Type I culture. This culture was of such virulence that a dilution of 2×10^{-9} caused the death of mice. The units of antibody per microgram were determined by the combining equivalent technic published elsewhere (20).

The results with the various preparations are shown in table 2. Significant differences were observed in these tests. The one of especial interest was the decrease in all biological activity of the control material with hot alkaline treatment (sample B) and the restoration to even greater activity than was found in the original material, by treatment of this sample (B) with NH_4OH, as shown in sample C. This was true for precipitable nitrogen, precipitins, and active immunity. Peculiarly enough, the combining equivalent was highest in the NH_4OH treated control (sample D), whereas the control (A) and sample C were practically the same. In contrast, the sample treated with hot NaOH (B) gave a higher combining equivalent than either A or C.

In the preparation treated with NH_4OH and then with NaOH (E), there was decreased activity, but not so much as occurred in the orig-

inal material treated with NaOH (B). In other words, the original material treated with NH_4OH has been in some way stabilized so that it has become resistant to treatment with hot NaOH, as shown by alteration in both chemical characteristics and biological activity. On the other hand, the combining activity of this sample, in terms of amount of antibody neutralized, was very much lower than that of any other preparation.

In addition, tests for relative immunizing activity of these five samples were carried out by injecting a group of mice with a single dose of the respective samples which had been found to produce

TABLE 2

Immunological study

SAMPLE	PRECIPITABLE ANTIBODY PROTEIN, MGM. N PER CC. SERUM				PRECIPITIN TITER	IMMUNITY IN MICE	UNITS PER MICROGRAM
	Concentration of samples						
	4×10^{-4}	2×10^{-4}	1.3×10^{-4}	1×10^{-4}			
A. P46 control material...	0.202	0.165	0.144	0.138	14.3×10^{-7}	2×10^{-8}	3.6
B. (A) treated with NaOH.....	0.112	0.077	0.066	0.059	1.0×10^{-4}	1×10^{-6}	5.4
C. (B) treated with NH_4OH.....	0.240	0.196	0.184	0.176	6.67×10^{-7}	1×10^{-8}	3.3
D. (A) treated with NH_4OH.....	0.214	0.177	0.176	0.170	4.0×10^{-7}	1×10^{-8}	8.4
E. (D) treated with NaOH.....	0.182	0.167	0.135	0.036	8.0×10^{-7}	4×10^{-9}	0.9

active immunity. The degree of immunity produced was measured by injecting groups of three mice with respectively 5, 50, 500, and 5000 lethal doses, seven days after the injection of antigen. As shown in table 3, the results are definite. The original material, which was biologically active (A), was changed to one of greatly reduced activity by treatment with hot NaOH (B), and restored to equal or even greater strength than in its original form A.

Now all of the alkaline treated samples, B, C, and D, contained in terms of acetyl groups roughly one-seventh of the original sample A. The one treated with hot NaOH (B) had a slightly higher percentage than the two NH_4OH treated fractions (C) and (D) possibly

due to splitting of terminal groups. And yet both of these latter were 99 per cent more active immunologically than the sample B which had been subjected to hot NaOH. Therefore, the work of Avery and Goebel in which they report that the active Type I immunising polysaccharide possesses its antigenic activity because of the presence of an acetyl group is not confirmed. On the other hand, with the sample treated with hot NaOH (B), which was a greatly altered substance, our results as to the destructive influence of alkali

TABLE 3

Relative immunizing activity against Type I organisms

SAMPLE	DILUTION OF SAMPLE	CULTURE DILUTIONS			
		10^{-4}*	10^{-5}	10^{-7}	10^{-8}
A. P46 control material	10^{-6}	20†	s	s	s
		24	s	s	s
		s	s	s	s
B. (A) treated with NaOH	10^{-6}	22	24	22	s
		22	24	36	s
		24	24	66	s
C. (B) treated with NH_4OH	10^{-6}	66	s	36	s
		s	s	s	s
		s	s	s	s
D. (A) treated with NH_4OH	10^{-6}	36	48	s	s
		70	s	s	s
		s	s	s	s

The supply of sample E was exhausted.

* Dilutions contain 5000, 500, 50, 5 lethal doses, respectively.

† Numbers refer to hours of survival; s indicates survival.

and heat on the Type I immunizing antigen confirm the findings of these investigators. However, the strongest evidence that in our preparations the acetyl, if present, had little significance on this biological characteristic was the fact that while heating with one alkali caused a decrease, subsequent treatment of this preparation with another alkali NH_4OH resulted in a restoration not only to its former activity but even to a greater one.

In our work on the immunizing antigen of the pneumococcus, it

has been repeatedly observed that some samples produce a heterologous response in white mice. With human beings this had been noted by Finland and Sutliff (21), and by Felton, Sutliff and Steele (22). Both Day (23) and Harley (24) have reported a heterologous response from a pneumococcus antigen in laboratory animals. In

TABLE 4

Relative immunizing activity against Type II organisms

SAMPLE	DILUTIONS OF TYPE II CULTURE								
	10^{-6} (500 L.D.*)			10^{-7} (50 L.D.)			10^{-8} (5 L.D.)		
	Dilutions of samples†								
	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}
A. P46 control material	s‡	36	36	s	s	36	s	s	s
	s	s	s	s	s	s	s	s	s
	s	s	s	s	s	s	s	s	s
B. (A) treated with NaOH	36	36	36	72	24	22	s	s	36
	36	60	36	s	36	36	s	s	s
	s	s	s	s	s	36	s	s	s
C. (B) treated with NH_4OH	36	22	36	s	36	36	60	36	36
	36	s	s	s	s	60	s	44	s
	s	s	s	s	s	s	s	s	s
D. (A) treated with NH_4OH	36	36	24	s	36	36	s	36	36
	36	36	36	s	s	36	s	s	s
	72	36	s	s	s	36	s	s	s
E. (D) treated with NaOH	s	s	22	s	36	36	22	s	36
	s	s	22	s	s	s	s	s	s
	s	s	s	s	s	s	s	s	s

* L.D. indicates lethal doses.

† Single injection of 0.5 cc. of the various dilutions.

‡ s indicates survival, numbers refer to hours of survival.

all our samples so far studied, this response followed injections of material in relatively high concentrations as compared with the amount necessary to stimulate specific response. The five samples made from specific Type I polysaccharide described in this report have all been tested in white mice for activity against Type II pneumococci. Any positive results followed the use of dilutions of be-

tween 10^{-4} and 10^{-6}. In one experiment mice were injected with 0.5 c.c. of 10^{-4}, 10^{-5}, and 10^{-6} dilutions of the samples in groups of 9 each, and on the seventh day afterward, triplicate mice from each group were injected with 5, 50, and 500 lethal doses respectively of Type II pneumococci. The results are given in table 4. Briefly, the control material was the most potent, showing an activity against Type II organisms as great as was found against Type I (see table 2). The other samples exhibited only slight activity. Sample E, however, the sample of the control treated with NH_4OH and then heated with NaOH, was almost as antigenic as the control. It is to be emphasized that, although the original material was specific for Type I in *in vitro* tests, that is, the precipitable nitrogen, the precipitin, and the combining equivalent tests, yet it produced in mice immunity against both Type I and Type II pneumococci.

DISCUSSION

The work reported here was done on a single sample of material. However, it has been found possible to duplicate the results with other preparations of polysaccharide, in that following heat treatment with N/10 NaOH, aldehyde groups were oxidized or otherwise removed, the optical rotation became zero, and the reactivity for both iodine and bisulfite was reduced to almost negligible levels. In other words, a specific modification of the polysaccharide can be reproduced.

The explanation of the alteration in optical activity and its correlation to biological manifestations must await further work. The important consideration now is the demonstrable reversibility of both the biological and the chemical activities. This includes mouse immunity, precipitable nitrogen, and precipitin titer.

The observations on the four samples prepared by alkaline treatment indicate that the polysaccharide is a labile molecule which is sensitive to strong alkali. In our opinion, the cause for the reduction of immunizing property is not de-acetylation but more probably some intramolecular rearrangement. In other words, immunizing activity of Type I polysaccharide for white mice is perhaps not due to "side chains," but to a basic molecular unit which is more or less resistant

to certain chemical agents, for instance, to acids, is greatly altered apparently reversibly by other chemical means, *i.e.*, strong alkalis, without postulating the possible active acetyl group.

Generally speaking, all antigens have been considered to be protein in nature. The hapten of Landsteiner (25), the "determinant" coupled with a protein, was called a "complete antigen." In the case of pneumococci this idea has been advanced especially by Heidelberger (26). However, since there is agreement not only that the pneumococcus polysaccaride actively immunizes animals against the living culture, but that antibody response follows its injection, the conception of the true structure of certain antigens needs revision. Landsteiner, in his recent book, agrees to the necessity of this change, but still points out the importance of the hapten as a "determinant" of the specificity of an antigen. He speaks of a hapten as possibly an "incomplete antigen."

Perhaps the first examples of non-protein antigens are the much questioned reports of Uhlenhuth (27) on gum arabic and allied substances, and that of Ford (28) on Rhus toxicodendron. Developments in recent years would indicate that these investigators undoubtedly were working with antigens at least containing insignificant amounts of protein.

In this connection there is particular interest in preparation C reported above. For this sample, heated in N/10 NaOH at 100°C. for 30 minutes, precipitated, and then treated with NH_4OH at 4°C. for 18 hours, was antigenic. Now it is well known that heating an alkaline solution of protein destroys its antigenicity. Hartley (29) has further shown that this destruction occurs even before hydrolysis has begun. Hence, in all probability the antigen C may be considered to be non-protein in nature.

Furthermore, inasmuch as the aldehyde groups are removed, the resulting antigen does not contain a complete sugar molecule. Hence, although the hexose configuration is not necessarily changed, this antigen is not only non-protein, but may be considered non-polysaccharide as well. Moreover, these preparations of immunizing antigen, B and C, may be conceived as polyhexuronic acids. Thus, although the final preparation may not be chemically identical, our

findings especially with sample C are a confirmation of our former report of a pneumococcus "antigen non-protein and non-polysaccharide in nature."

SUMMARY AND CONCLUSIONS

A method has been indicated by which the linkage between the units in the polysaccharide of Type I pneumococcus are altered with concurrent changes in biological activity. This alteration is shown by both the high titer in bisulfite and iodine reactions in the original material and by the absence in samples B and C of reducing sugars on hydrolysis after destruction of the aldehyde groups in hot NaOH solution.

From this altered SSS, four samples were prepared by various alkaline treatments and tested both chemically and biologically in comparison with the original material. Each of the samples may be considered separately. The original material (A) gives tests for aldehyde groups, is high in "acetyl" content, and is optically as well as immunologically active. In B, a portion of A treated for 30 minutes in N/10 NaOH at 100°C., the optical activity is lost, the aldehyde groups are removed, "acetyl" content and all immunological activities are greatly reduced. In C, sample B treated with NH_4OH, there is a reduction of acid from vacuum distillation, no indication of sugar on acid hydrolysis, but an increase of the iodine reaction. Optical rotation is changed from zero to $+190°$, or higher than the control. Immunologically also this sample is more active than the original material (A). Sample D is the original material (A) treated with NH_4OH. There is a decrease of 90 per cent in acid on vacuum distillation, a slight decrease in glucose number, a higher optical rotation than in the original material, as well as a high titer in all immunological tests. It is significant that with a 90 per cent decrease in "acetyl" content the immunizing activity is higher than the original. There is also a significant increase in its combining equivalent with antibody. Sample E is the NH_4OH treated control (D) further treated with hot NaOH as in sample B. The NH_4OH treatment caused a rearrangement of the molecule or stabilization, for on acid hydrolysis glucose number was 10.25 as compared to zero with sample B. Optical activity was reduced but not entirely lost. Im-

munological tests, although decreased, were not as low as in sample B, with the exception of the combining equivalent and that was the lowest of all samples, 0.9 unit. The acid from vacuum distillation was higher than in sample D, indicating a splitting off of the terminal carboxyl radicals.

At this stage of our investigation certain inferences may be made: (1) An antigen has been prepared from a Type I polysaccharide which in our opinion is non-protein in nature for the following reasons: (a) treatment with hot NaOH (N/10) and then NH_4OH results in a product more highly antigenic than the original preparation, and (b) all well-recognized protein tests are negative including a test for sulfur with a large sample of material. (2) This antigen may be considered non-polysaccharide, since both the Molisch test and the test for reducing sugars after acid hydrolysis are negative. (3) The antigens so prepared from Type I SSS produce active immunity in mice against both Type I and Type II pneumococci. (4) The restoration of the biological properties destroyed with hot NaOH by treatment with NH_4OH shows that the "acetyl" content is of no significance in determining the biological activity of the preparation studied, but conversely indicates that this property is determined by a definite molecular configuration which is readily altered by strong alkalis.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Clinical Parasitology and Tropical Medicine. By DAMASO DE RIVAS and CARLOS T. DE RIVAS. Pp. 367, 144 text-figures, and one colored plate. $5.00. (*Lea and Febiger, Philadelphia, 1935.*)

This book is designed, according to the authors (p. 5), "to present the modern conception of tropical and parasitic diseases from the standpoint of etiology, the complete life history of parasites, the pathology, symptomatology, diagnosis, and treatment of these affections." Unfortunately, this thoroughly desirable object is not realized in the pages that follow. While the general fields of parasitology and tropical medicine are covered with varying degrees of emphasis, accuracy, and completeness, it cannot, in the opinion of the reviewer, be urged that the presentation is a modern conception in the sense that it makes a reasonable attempt to include recently determined knowledge or to exclude older beliefs that have been discredited. The book is a marvelous collection of misspellings, grammatical errors, incorrectly used scientific names and terms, antiquated figures, misstatements and omissions of facts.

The contents are divided into six parts: General Considerations, Diseases caused by Protozoa, Diseases caused by Metazoa, Diseases caused by Bacteria, Diseases of Undetermined Etiology, and Climatic Diseases, including Animal Poisons. Aside from the general features of the text, only the sections dealing with parasitic protozoa and protozoan diseases can be specifically criticized by the reviewer. The following notations indicate the type and scope of the errors both of commission and of omission. The number of protozoan species occurring in man is given as "50 or more" (p. 14), whereas the number usually recognized by protozoologists is under 30, the mosquito is incorrectly referred to as the "intermediate host" on the seventh line and, correctly, as the "primary host" of the malarial parasites on the seventeenth line of p. 15; illustration of cystic and precystic "forms of ameba from the discharge of pyorrhea alveolaris" (p. 76), "spore formation (schizogony)" of *E. histolytica* (p. 77), inadequate consideration of *E. coli* and no consideration of other non-pathogenic amoebae of man to be excluded in the recognition of *E. histolytica* (Chap. VI), no mention of carbarsone and vioform as amoebacidal drugs (Chap. VI), "emetine *hydrochlorate*" (p. 84) instead of the *hydrochloride*, the persistence of amoebic liver abscess "for months or years without producing subjective or objective symptoms" (p. 86), the reproduction of *Balantidium coli* by "conjugation" (p. 88), the assumption (which *may* be true but which has not yet been proved to be true) that Giardia, Trichomonas, and Chilomastix are causes of diarrhea and associated gastrointestinal disturbances

(p. 91), the occurrence in Giardia of "two parabasal bodies anteriorly, each containing a nucleus" (p. 92), the description of the human intestinal trichomonads as a single species with three anterior flagella (p. 95), the indiscriminate use of "gamete" and "gametocyte" (Chap. VIII) in reference to the sexual stages of the plasmodia of man, "dimorphic" *P. vivax* (p. 102), the statement that "the malarial treatment of paresis is still an empirical procedure which thus far is in the experimental stage" (p. 122), failure to mention use of Paris green or of drainage as malaria control measures (p. 125), the statement that "Tryparsamide is the newest preparation recommended for the treatment of African sleeping sickness" (p. 132), whereas there have been many, including Bayer 205, offered since tryparsamide was developed, the reference to *Leishmania donovani* as "a binucleated flagellate protozoa" (p. 139), the statement, entirely without basis, that "Recently Napier and his associates have demonstrated the transmission of kala-azar by *Phlebotomus argentipes*, and that according to other investigators the disease, in China, is transmitted also by a sandfly, *P. major*" (p. 140), the omission of any mention of the various biochemical tests in the diagnosis of kala-azar (Chap. X), the "multiple fission" of leishmanial organisms (p. 145), etc. This list is by no means comprehensive but it is representative. A much longer and more inclusive list (over eight pages) appears in Faust's review of the same book (1935, Am. J. Trop. Med., 15: 705–714).

J. A.

Immunology. By NOBLE PIERCE SHERWOOD. 608 pp., illus., $6.00. (*C. V. Mosby Co., St. Louis, 1935.*)

According to the author's preface, the book was ". . . written for medical students and for others who have had training in bacteriology, inorganic and organic chemistry, and who are interested in the underlying principles involved in infection and resistance, and diagnostic laboratory tests."

The content is an orderly presentation of the phenomena of immunology. The chapters give consideration to the logical divisions of the subject, such as humoral and cellular immunity, antibodies of the several varieties, antigens, antigen-antibody processes, blood grouping, complement fixation, serological syphilis, hypersensitiveness, and allergy.

The author utilizes, within the body of the chapters, headings of bold-faced type to designate the immediately following subject matter, which is definitive or descriptive, or both. Although many of the descriptions are lucid, adequate, and include the results of the most recent investigations, others are—possibly because of the condensation required by space—so brief as to be insufficient and are somewhat stereotyped. The apparent attempt at simplification of complicated subjects sometimes results in understatement. There is, however, a good bibliography at the end of each chapter.

The technique of bacterial complement fixation, and complement fixation and precipitin tests for syphilis are presented in more detail than other laboratory procedures.

The book will probably find more favor with those beginning the subject than with advanced students.

W. S. T.

Agents of Disease and Host Resistance. By FREDERICK P. GAY. 1581 pp., illus., $10.00. (*Charles C. Thomas, Springfield, Ill., 1935.*)

This book of more than 1,500 pages, by Dr. Frederick P. Gay and his associates, is a large single volume which presents the accumulated and, in most instances, current information concerning living agents in association with disease. The subject matter is unusually comprehensive and the scope is broadened beyond the bounds which often delimit books on the subjects of infection and resistance. In addition to a detailed survey of bacteriology and immunology, parasitology and protozoölogy are included, as well as a brief consideration of inanimate objects in relation to disease as exemplied by chemical poisons and tolerance to drugs.

Approximately the first third of the book is devoted to more general topics, including history, morphology, physiology, epidemiology, and the biological and chemical aspects of cellular and humoral phenomena. The subsequent chapters describe specific pathogenic agents in detail and their special relationships to the diseases with which they are associated. These sections contain descriptions of bacteria, spirochetes, fungi, rickettsia, viruses, protozoa, helminths and arthropods. Finally, the practical application of the knowledge to diagnosis, therapy and prevention of infectious diseases, is presented. The subjects not immediately related to living agents are dealt with briefly.

The outline just given indicates the generous amount of information contained between the covers of this volume. The enlarged point of view is, for the most part, a desirable feature of the book, in that it calls attention to certain common denominators and contrasts among the several subjects. The authors have even gone farther in including diversified subject matter and have devoted one chapter to a variety of unrelated ailments, such as epilepsy and hypertension, and others that belong to the domains of endocrinology or psychiatry. The cursory and paragraphic comments are uninformative concerning diseases of great importance in their own right.

The book, as a whole, is complete and the style, in general, is readable and lucid. There is an extensive bibliography at the end of each chapter. Although the subject matter is brought up to date on most topics, in a few instances there is a lag of several years in the presentation of current literature. The defects are, however, minor and one may expect the volume to take its place among standard works on the subject of living agents and disease.

W. S. T.

Diseases of Women. HARRY S. CROSSEN and ROBERT JAMES CROSSEN. 8th Ed., Revised, 999 pp. Illustrated. $10.00. (C. V. Mosby Company, St. Louis, 1935.)

A revision of one of the most popular students' textbooks on Gynecology,

containing new material and illustrations and bringing all subjects up to date in accord with recent literature.

A short but comprehensive section on the most recent developments in the field of hormones has been added and glandular therapy is discussed in the treatment of all conditions in which it is warranted.

The chapter on sterility has been brought up to date and enlarged upon. A concise chapter on gynecological pathology has been added.

The book is profusely illustrated and the cuts are unusually clear and graphic. Many new illustrations have been added in this edition. There is one serious fault; the authors have failed to include a bibliography.

Although loosely and carelessly written in places, it will probably continue to be the outstanding one volume textbook on Gynecology.

J. H. L.

Haemochromatosis. By J. H. SHELDON. 382 pp. 40 illus. $8.50. (*Oxford University Press, London, 1935.*)

This book represents a model of meticulous reporting. The world's literature on haemochromatosis is spread before the reader, not merely by citation of title, but sufficiently by content. Every speculation on the genesis and significance of the disease is here recorded. The reader obtains, almost literally, a view of every patient who has been reported—and of postmortem and biochemical studies, if they were made. One need have no interest in haemochromatosis, as such, to be fascinated by this monograph—so overwhelming is the mass effect of the display of studious observation, and ingenious reasoning on the inner mysteries of pigment metabolism.

The author attributes the disease to a universal disturbance of the intracellular metabolism particularly concerned with the intracellular phase of respiration. This anomaly is perhaps congenital. He denies Mallory's belief that copper is an etiological agent, though he thinks that this metal may have something to do with the disease-process.

S. W.

BOOKS RECEIVED

The Single, the Engaged, and the Married. By MAURICE CHIDECKEL. 268 pp. $2.50. Eugenics Publishing Co., New York, 1936.

Medical Papers. Dedicated to Henry Asbury Christian. Edited by Robert T. Monroe. 1000 pp. Waverly Press, Baltimore, 1936.

Prostitution in the Modern World. By GLADYS M. HALL. 200 pp. $2.00. Emerson Books, Inc., New York, 1936.

Physiology of Love. By PAOLO MANTEGAZZA. Translated from the Italian by Herbert Alexander. Edited by Victor Robinson. 237 pp. $3.00. Eugenics Publishing Co., New York, 1936.

Pediatric Nursing. By JOHN ZAHORSKY. 568 pp., illus. $3.00. C. V. Mosby Co., St. Louis, 1936.

Psychology of Sex. By HAVELOCK ELLIS. 377 pp., $3.00. Emerson Books, Inc., New York, 1935.